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Effect of combination of ultraviolet light and hydrogen peroxide on inactivation of *Escherichia coli* O157:H7, native microbial loads, and quality of button mushrooms

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ABSTRACT

Mushrooms are prone to microbial spoilage and browning during growing and processing. Ultraviolet light (254 nm, UV-C) has been used as an alternative technology to chemical sanitizersfor food products. Hydrogen peroxide (H_2O_2) is classified as generally recognized as safe for use in foods as a bleaching and antimicrobial agent, and could control the bacterial blotch and browning of mushrooms. This study investigated the effects of water wash (control), 3% H_2O_2 wash, 0.45 kJ m^{-2} UV-C, and combination of H_2O_2 and UV-C (H_2O_2 + UV) on microbial loads and product quality of mushrooms during storage for 14 days at 4 °C. Additionally, the inactivation of *Escherichia coli* O157:H7 inoculated on mushrooms was determined. Results showed that water wash, H_2O_2, UV-C and H_2O_2 + UV resulted in 0.44, 0.77, 0.85, and 0.87 logs CFU g^{-1} reduction of *E. coli* O157:H7, respectively. Hydrogen peroxide, UV-C and the combination reduced total aerobic plate counts on the surface of mushrooms by 0.2–1.4 logs CFU g^{-1} compared to the control, while there was no significant difference among the three treatments during storage. After storage, UV-C treated mushrooms had similar L^* and a^* values as the control while H_2O_2 and H_2O_2 + UV-C treated mushrooms had higher L^* (lighter) and lower a^* (less brown) values than the control. Compared to water wash, all the treatments inhibited lesion development on the mushroom surface on day 14. The combination of H_2O_2 and UV achieved the best overall dual control of lesion and browning. There was no significant difference in ascorbic acid and total phenolic content among the treatments. Overall our results suggested that H_2O_2 + UV reduced microbial loads, and extended storage life by reducing lesion development without causing deterioration in nutritional quality of button mushrooms. Therefore, when properly utilized, H_2O_2 + UV could potentially be used for maintaining postharvest quality while marginally reducing populations of *E. coli* O157:H7 and background microflora.

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1. Introduction

Because of their nutritional value and other functional properties, mushrooms are consumed around the world. The cultivated button mushroom (*Agaricus bisporus*) is the most common edible mushroom. However, mushrooms can be easily contaminated with microorganisms, and are prone to spoilage and browning during growing and processing (Simon, Gonzalez-Fandos, & Tobar, 2005).

Different methods based on washing to extend storage time and reduce the contamination levels on mushrooms have been developed (Pramod, Fernanda, Anthony, & Aurelie, 2008; Yuk, Yoo, Yoon, Marshall, & Oh, 2007). However, some washing treatments would result in tissue injury of mushrooms because of the mechanical damage and increased water uptake during the washing operations. For example, mushrooms washed with hypochlorite at 50 ppm generally deteriorate more rapidly than unwashed due to mechanical damage and water uptake, which in turn, leads to subsequent bruising and increased microbial growth (Cliffe-Byrnes & O'Beirne, 2008). Among the washing methods, it is reported that mushrooms washed with H_2O_2 were free of adhering compost or casing residues, less subject to brown blotch than conventionally

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washed mushrooms (Sapers, Miller, Choi & Cooke, 1999; Sapers, Miller, Pilizota & Kamp, 2001), and washing mushrooms for 60 s with 3% H_2O_2 produced best quality and were superior to ClO_2 in reducing *pseudomonad* counts (Cliffe-Byrnes & O'Beirne, 2008). H_2O_2 is classified as GRAS (generally recognized as safe) in USA for use in food products (Sapers & Simmons, 1998).

As a nonthermal and environment-friendly technology, ultraviolet C (UV-C) radiation has been approved for use as a disinfectant for surface treatment of food (US-FDA, 2002) and is used for microbial reduction in food products. In addition, UV-C can cause various biochemical responses in fresh produce including induction of antifungal enzymes and formation of phytoalexin compounds (Shama, 2007). Jiang, Jahangir, Jiang, Lu, and Ying (2010) reported that UV-C could delay softening and enhance antioxidant capacity in shiitake mushrooms. In addition, irradiation of button mushrooms with UV-C could inhibit lesion development on the mushroom surface, reduce *Escherichia coli* O157:H7 and total aerobic plate counts on mushroom cap surfaces and result in a significant increase in vitamin D_2 content (Guan, Fan, & Yan, 2012; Teichmann, Dutta, Staffas & Jagerstad, 2007). Therefore, it has been proposed that UV-C light be used for sanitizing fresh button mushrooms and extending shelf-life.

A combination of H_2O_2 with UV-C, based on the principle of advanced oxidative process, has been developed with enhanced efficacy for inactivating bacteriophage on lettuce surfaces (Xie, Hajdok, Mittal & Warriner, 2008). Hadjok, Mittal, and Warriner (2008) concluded that UV + H_2O_2 could reduce the populations of human pathogens and spoilage bacteria on the surface and those internalized within fresh lettuce, spinach, broccoli, cauliflower, onion and tomato without affecting the shelf-life stability. To our knowledge, none of the studies examined the efficacy of UV-C in combination with H_2O_2 on microbial and sensory quality of mushrooms during cold storage.

The objective of this work was to evaluate the effects of H_2O_2 combined with UV-C radiation on inactivation of *E. coli* O157:H7 and the microbial loads on mushrooms during cold storage. In addition, visual quality, total phenolics and ascorbic acid contents of mushrooms were examined.

2. Material and methods

2.1. E. coli inactivation

2.1.1. Strains and culture media

Three isolates of *E. coli* O157:H7 were selected from the culture collection maintained (−76 °C) at the USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA: 1) *E. coli* O157:H7 USDA/FSIS 45753-35; 2) *E. coli* O157:H7 strain 933; and 3) *E. coli* O157:H7 strain A9218-C1. The culture and the harvest of strains were carried out according to the procedure described by Guan, Fan, and Yan (2012) and Huang (2010). *E. coli* O157:H7 was cultured independently in 0.01 L Brain Heart Infusion Broth (BHI, BD, Sparks, MD) at 37 °C (at 200 rpm) for 18 h, and each culture was harvested by centrifugation (2400× *g* for 10 min at 4 °C), washed twice with 0.01 L 1 g L^{-1} peptone water (PW, BD, Sparks, MD). The cell pellets were re-suspended and combined as a cocktail.

2.1.2. Inoculation of mushroom

Mushrooms (*A. bisporus*) were purchased from local supermarket, and stipes were removed with a stainless steel blade before inoculation. Each mushroom was spot inoculated on the cap surface by placing 4×10^{-5} L of the three-strain mixture of *E. coli* O157:H7 using a micropipette in a laminar flow biological hood (Model MU-425-600, NuareTM, Plymouth, MN, USA). The inoculated mushrooms with the caps facing up were air-dried for 1 h in the hood before H_2O_2 treatment and UV-C irradiation.

2.1.3. UV-C/H_2O_2 treatment

Four inoculated mushrooms were dipped in water (CK) or 3% H_2O_2 in a 2 L beaker for 30 s at ambient temperature (~23 °C) and with gentle agitation, and were then treated with or without UV-C. UV-C irradiator containing four 0.61-m UV-C emitting bulbs (SaniLIGHT™, Atlantic Ultraviolet, White Plains, NY, USA) was used, and irradiation was carried out with the mushroom caps facing the UV-C lamp (about 0.20 m, 30 W m^{-2}) for 15 s (0.45 kJ m^{-2}). The UV-C dose rate was determined by UVX Digital Radiometer (Serial No. E28298, UVP, Inc., Upland, CA, USA). The tests were repeated 4 times.

2.1.4. Microbial analysis

Determination of *E. coli* O157:H7 population was carried out as described by Guan et al. (2012). Four mushrooms was blended with 0.08 L 1 g L^{-1} sterile peptone water in stomacher bags and massaged at the inoculated cap surface by hand for 1 min. Serial dilutions were prepared in 9 mL 1 g L^{-1} PW. From each dilution, 1×10^{-4} L aliquots were withdrawn and surface-plated onto Sorbitol-MacConkey Agar (SMAC, BD, Sparks, MD, USA) plates, and the SMAC plates were incubated at 37 °C for 24 h, and typical colonies of *E. coli* O157:H7 were counted. All microbial counts were reported as CFU g^{-1}values.

2.2. Effect of UV-C/H_2O_2 on quality

2.2.1. Preparation of mushroom

Mushrooms were obtained from Phillips Mushroom Farms (Kennett Square, PA, USA). Immediately after harvest, the mushrooms were transported to a produce processing laboratory at USDA, ARS, ERRC, where they were stored at 4 °C for no more than 2 d before treatments. Mushrooms without obvious bruising or other damage and with cap sizes of 4–5 cm (in diameter) were chosen and treated at ambient temperatures (~23 °C).

2.2.2. H_2O_2 and UV-C treatment

Ten mushrooms were dipped for 30 s in distilled water or 3% (W/V) H_2O_2, and were then drained by a stainless steel mesh strainer (NORPRO®, Everett, WA, USA) and directly packaged or treated by 0.45 kJ m^{-2} UV-C irradiation applied to both sides of mushrooms (H_2O_2 + UV). The UV-C dose (0.45 kJ m^{-2}) was selected based on our preliminary experiments that doses higher than 0.45 kJ m^{-2} did not further increase the reduction of *E. coli* inoculated on the surface of mushrooms (Guan et al., 2012). The treated mushrooms were placed into a rigid plastic container (ClearPAC®, C24DER, 24OZ, Dart Container Corp., Mason, MI, USA) with a lid (C32DLR) perforated with 4 holes (6×10^{-4} m in dia.). The packaged mushrooms were then stored at 4 °C until analysis. At 1, 7, 14 d of storage, color, microbial population, presence of lesions, and nutritional properties of mushroom were measured. For preparing samples for the analysis of total phenolics and ascorbic acid, 6 mushrooms from each replicate were cut into thin slices (~0.2 cm in thickness) and mixed thoroughly. And then aliquots (0.025 kg) of mushroom slices were frozen in liquid nitrogen and stored at −80 °C prior to extraction and determination of nutritional properties. Each treatment was conducted independently 4 times ($n = 4$) on the same day.

2.2.3. Analysis of total microbial loads

Four mushrooms were transferred to sterile stomacher bags with1 g L^{-1} sterile PW equal to sample weight. After massaged by hand for 1 min, serial dilutions were prepared in 9 mL 1 g L^{-1} PW.

Then 1×10^{-4} L aliquots of each of the dilutions were pour-plated onto on tryptic soy agar (TSA) and incubated at 35 °C for 48 h. Microbial counts were reported as CFU g^{-1}values.

2.2.4. Color analysis

Color analysis for the cap surface of mushrooms was performed using a ColorQuest XE colorimetric spectrophotometer (Hunter Associates Laboratory, Reston, VA, USA). Results were from 4 independent experiments, with 4 readings taken for four mushrooms in each treatment per experiment.

2.2.5. Lesion evaluation

Before the analysis of other quality parameters, severity of lesions on the surface of mushrooms was estimated based on appearance using a scale of 1–5, where 5 = severe lesions and 1 = none (Sapers, Miller, Pilizota & Kamp, 2001). For each replicate sample, samples were randomly evaluated by three-member trained panelists who were familiar with the scoring system.

2.2.6. Total phenolics analysis

Samples (0.025 kg) were homogenized with 0.05 L of 80% methanol using a homogenizer (Virtishear, Virtis, Gardiner, NY, USA) at a speed setting of 70 for 4 min, and the homogenate was filtered through 4 layers of cheesecloth and then centrifuged at 10,000× *g* for 10 min at 5 °C. Supernatants were stored in a −80 °C freezer and analyzed for phenolic content within 3 weeks of storage. Total phenolic content was measured using the Folin–Ciocalteu colorimetric method (Fan, 2005; Guan et al., 2012). There were 4 measurements for each sample from one replicate, and there were a total of 16 measurements for each treatment.

2.2.7. Ascorbic acid content analysis

The determination of ascorbic acid (AA) was carried out as described by Fan, Annous, Beaulieu, and Sites (2008). Mushroom tissues (0.025 kg) were blended with 0.05 L 62.5 mM metaphosphoric acid (MPA) in a homogenizer, and centrifuged at 12,000× *g* for 600 s at 5 °C. The supernatant was analyzed using a Hewlett Packard Ti series 1050 HPLC system (Agilent Technologies, Palo Alto, Calif., USA) at 254 nm, and AA was calculated from an external standard curve. There were two measurements for each replicate of sample, and there were a total of 8 measurements for each treatment.

2.3. Statistical analysis

Experiments were arranged in a completely randomized design, and each treatment was composed of four replicates. The least significant difference (LSD) test was performed using SAS 9.2 (SAS Institute Inc., Raleigh, NC, USA). Calculations of means and standard deviations were performed by Microsoft Excel 2003.

3. Results and discussion

3.1. Inactivation of E. coli and microbial loads of button mushrooms during storage

The effects of 3% H_2O_2, 0.45 kJ m^{-2} UV-C and the combination on the population of *E. coli* O157:H7 inoculated on mushroom are presented in Table 1.

The population of *E. coli* inoculated onto the four mushroom surfaces was 7.14 ± 0.27 log CFU g^{-1}, and water dipping (control) achieved 0.44 ± 0.14 log CFU g^{-1} reduction of *E. coli*. Compared with the control, H_2O_2, UV and H_2O_2–UV significantly ($P < 0.05$) reduced the population of *E. coli* by 0.33, 41 and 0.43 log CFU g^{-1}, respectively. The log count reduction in *E. coli* population by H_2O_2 was not significantly different from that achieved using UV-C or H_2O_2 + UV.

Table 1
E. coli O157:H7 population reduction on inoculated mushrooms after H_2O_2/UV treatment.

Treatment	Reduction (log CFU/mushroom)[a]
CK	0.44 ± 0.14 a
H_2O_2	0.77 ± 0.27 b
UV-C	0.85 ± 0.27 b
H_2O_2 + UV	0.87 ± 0.28 b

[a] Initial counts of *E. coli* O157:H7 on the four mushrooms were 7.14 ± 0.27 log CFU (mean ± standard deviation). Data followed by different letters in the same column are significantly ($P < 0.05$) different among the treatment.

The disinfection mechanism of H_2O_2 is based on the release of free oxygen radicals. Free radicals have both oxidizing and disinfecting/bleaching abilities (Block, 1991). At the same time, it may also be due to better removal power caused by the physical force of effervesce on the mushroom surface (Cliffe-Byrnes & O'Beirne, 2008). UV-C causes DNA strand breakage in bacterial, yeast, and human cells, which results in the inhibition of DNA replication (Sommers & Cooke, 2009). The most important factors affecting the lethality of UVC include: the equipment and treatment parameters, the physicochemical characteristics of the media and the type of microorganisms (Gayan, Monfort, Alvarez, & Condon, 2011). It is thought that the synergistic action of H_2O_2 and UV combination for bacterial inactivation is based on the principle of advanced oxidative process, whereby highly reactive, but short-lived, hydroxyl radicals are formed during the interaction of H_2O_2 with UV photons (Rosenfeldt, Linden, Canonica & Gunten, 2006). Hadjok et al. (2008) found that the reduction of *Salmonella* spp. on and within lettuce by the combination of H_2O_2 (50 °C, 1.5%) and UVC (37.8 mJ cm^{-2}) was significantly higher compared with the individual treatments. However, our present study demonstrates that no more than 1 log reduction of *E. coli* O157:H7 was achieved on mushrooms, and the combination did not result in significant greater reduction of *E. coli* compared with single treatment. Crowe, Bushway, Bushway, Davis-Dentici, and Hazen (2007) has also reported that a combination of UV and H_2O_2 did not result in a significant decrease in the bacterial numbers associated with blueberries compared with that when H_2O_2 was applied alone. It is possible that the lack of an observed synergistic effect was a consequence of treatment of H_2O_2 at ambient temperature, where free radicals generated would be lower or short lived (Hadjok et al., 2008). H_2O_2 and UV showed no synergies against *Enterococcus faecalis* and coliphage MS2 virus in wastewater-like test medium (Koivunen & Heinonen-Tanski, 2005). The ineffectiveness of H_2O_2 and UV in reducing *E. coli* on mushroom observed in the present study may be due to the porosity of mushroom surface and subsequent infiltration of *E. coli* O157:H7 cells into the mushroom. The internalization would protect the *E. coli* O157:H7 from exposure to H_2O_2 or UV. Consequently, a more penetrating form of treatment would be required to inactivate the pathogen.

3.2. Effect of H_2O_2/UV-C on microbial loads of button mushrooms during storage

To examine the effect of H_2O_2/UV on the proliferation of mushroom native bacterial populations during refrigerated storage, a 14 day storage study was performed after mushrooms were treated with 3% H_2O_2, 0.45 kJ m^{-2} UV-C and H_2O_2 + UV. The changes in TAPC after treatment of mushrooms during storage at 4 °C are shown in Fig. 1.

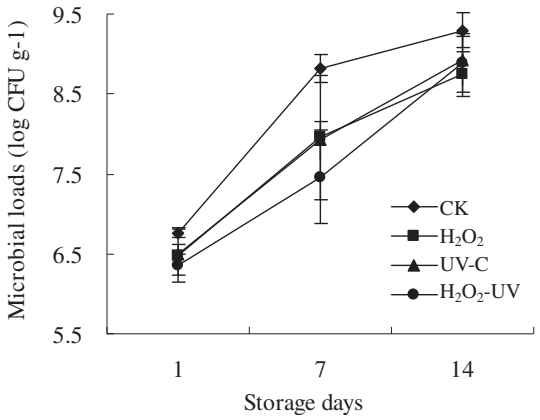

Fig. 1. Effect of H_2O_2/UV-C treatments on TAPC of button mushroom stored for 14 days at 4 °C. Vertical bars represent standard deviation ($n = 4$).

The TAPC for all treatments increased with storage time, and the control samples showed the higher TAPC (6.75–9.29 log CFU g^{-1}) than those of the treatments (6.30–8.30 log CFU g^{-1}). Compared to CK, H_2O_2 showed reductions of 0.27, 0.86, 0.54 log CFU g^{-1}, UV-C showing reductions of 0.25, 0.89, 0.38 log CFU g^{-1}, and H_2O_2 + UV showing reductions of 0.24, 1.36, 0.41 log CFU g^{-1} on day 1, day 7 and day 14, respectively. These results indicated that H_2O_2 treatment had similar effect as 0.45 kJ m^{-2} UV for reducing bacterial populations, and the combined treatment was more effective than single treatment on day 7, although there was no significant difference among the three treatments during storage. Because of the rapid breakdown of H_2O_2 due to mushroom catalase, it is unlikely that the storage effects were due to residual H_2O_2. It is more likely that the microbial reductions in treated samples during storage were due to UV and/or H_2O_2–induced injury to the microflora.

It has been stated that bacterial populations and factors that influence their growth play an important role in the postharvest quality of mushrooms (Soler-Rivas, Jolivet, Arpin, Olivier, & Wichers, 1999). High initial total aerobic bacteria counts about 7 log CFU on mushrooms were reported, and there was an increase of about 1.5 log units after 7 days storage at 4 °C (Cliffe-Byrnes & O'Beirne, 2008; González-Fandos, Olarte, Gimenez, Sanz & Simon, 2001). Similar results were found in the present study showing a 6.75 log CFU g^{-1} initial counts of total aerobic bacteria, and an increase of 2.54 log CFU g^{-1} during 14 days storage at 4 °C. Guan et al. (2012) reported that the initial TAPC was reduced by about 0.89–0.63 log CFU g^{-1} on the surface of mushrooms by 0.45 kJ cm^{-2} UV-C radiation treatment, and TAPC of mushrooms generally decreased with increasing UV-C dose. It is also reported that use of H_2O_2 (3% and 5%) on whole mushrooms prior to slicing could achieve greater than 1-log reductions for *pseudomonad*, mesophilic and psychrophilic bacterial populations and improved keeping quality during 19 days storage at 4 °C (Brennan, Port & Gormley, 2000; Cliffe-Byrnes & O'Beirne, 2008).

3.3. Effect of H_2O_2/UV-C treatments on color and lesions of button mushroom during storage at 4 °C

Color change on the surface of mushroom is an important factor which affects the visual appearance of mushrooms. Lower L^* indicates darkening of the mushroom while increasing a^* shows increasing redness, and an increase in b^* value suggests increasing yellowness of the mushrooms (Du, Fu, & Wang, 2009). The effects of H_2O_2, UV-C, and the combination treatments on L^*, a^* and b^* values of mushrooms are shown in Fig. 2.

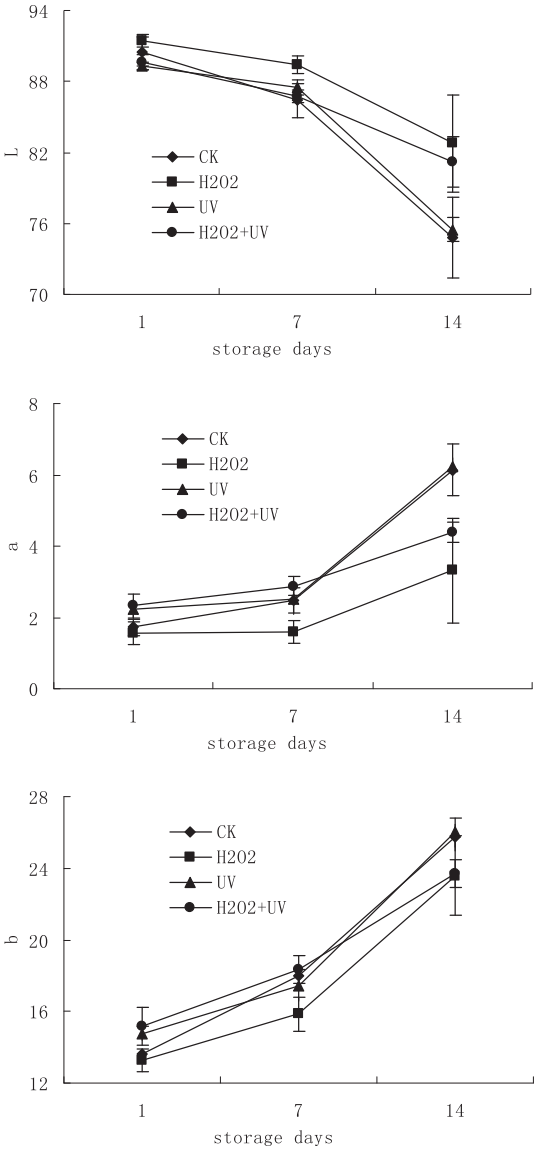

Fig. 2. Effect of H_2O_2/UV on L^*, a^* and b^* values of button mushroom stored for 14 days at 4 °C. Vertical bars represent standard deviation ($n = 4$).

L^* values of mushrooms decreased during storage, while a^* and b^* values of all samples tended to increase with increasing storage time. Mushrooms had higher L^* values and lower a^* and b^* values at the beginning of storage, and the color changed slowly before day 7. After 7 days, the L^* values began to decline significantly, and the a^* and b^* values increased significantly. H_2O_2 was the most effective in preserving the color of mushrooms compared to any other treatment during the entire storage period. The color of mushrooms treated by UV and H_2O_2 + UV was slightly darker than the control on day 1. Smaller changes in L^*, a^* and b^* of mushrooms during cold storage were observed for the UV and H_2O_2 + UV treated samples compared to the control during the first 7 days of storage. At the end of storage (day 14), the control mushrooms achieved the lowest L^* value and highest a^* and b^* values among all treatments, and UV-C treated samples didn't show significantly differences compared to the control, while the combined treatment of H_2O_2 and UV-C showed significantly higher L^* and lower a^* and b^* than UV-C and controls. After 14 days of storage, there was no significant difference in the L^*, a^* and b^* values of the mushroom treated with H_2O_2 and the combined treatment. H_2O_2 could release free oxygen

radicals that have both oxidizing and bleaching abilities. In addition, the radicals may have anti-browning capabilities as they could inactivate mushroom tyrosinase which is the major PPO enzyme responsible for browning in mushrooms (Cliffe-Byrnes & O'Beirne, 2008). However, UV-C could induce tissue damage to the mushroom cap surface tissue immediately after irradiation, which counteracted the ability of H_2O_2 in keeping the color of mushrooms. These results agree with previous reports on lettuce, baby spinach and mushrooms where higher UV-C doses caused cell damage or browning (Escalona, Aguayo, Martínez-Hernández & Artés, 2010; Guan et al., 2012).

The score of dark brown spots (lesions) on the surface of mushrooms was assessed during storage. On day 7, there was no lesion development on the surface of treated mushrooms, while the lesion score of the control samples washed with water was significantly higher than other treatments (Fig. 3). On day 14, lesions appeared on the surface of all the mushrooms, and there were significant ($P < 0.05$) differences among the treatments. It demonstrated that the combined treatment of H_2O_2 and UV achieved the best overall control of lesion development on the surface of mushrooms, and H_2O_2 was more effective than UV-C. The development of lesions on mushroom caps during storage is due to *Pseudomonas tolaasii* (Soler-Rivas et al., 1999), leading to activation of polyphenol oxidase and oxidation of phenolic compounds to form melanins.

Both UV-C and H_2O_2 can inactivate some bacteria including *P. tolaasii* on the surface of mushrooms, which could result in less dark brown spots developed on mushroom (Guan et al., 2012; Sapers & Simmons 1998). Although UV-C and H_2O_2 had no significantly synergistic effect on inactivation of bacteria on mushrooms during storage, the combination had a synergistic effect on inhibition of the lesion development, which may be attributed to the hormesis induced by UV-C to elicit a range of biochemical responses in fresh produce ranging from production of phytoalexin compounds to delays in ripening (Shama, 2007). Studies are needed to explore the mechanism of the combination of H_2O_2 and UV-C on lesion development of mushrooms besides inactivation of organisms.

3.4. Effect of H_2O_2/UV-C treatment on total phenolics and ascorbic acid of button mushrooms

Little is know about the effects of combination of UV and H_2O_2 on the total phenolic and ascorbic acid contents of button mushrooms during cold storage. Total phenolic and ascorbic acid contents of mushrooms increased during the 14-d period storage both in the controls and in samples subjected to the H_2O_2/UV-C treatments (Table 2). It was reported that phenolic compounds in plant materials can be present as a covalently bound form with insoluble polymers (Peleg, Naim, Rouseff, & Zehavi, 1991). Therefore, it is suggested that the bounded compounds may be liberated from insoluble portion of mushroom during storage, which, in turn, increases the pool of bioaccessible antioxidant compounds (Choi, Lee, Chun, Lee, &Lee, 2006). The reasons for the increases in phenolics and ascorbic acid contents of button mushrooms during storage are not clear. Perhaps, loss of moisture during storage partially contributed to the increases. The exact mechanisms need to be further studied.

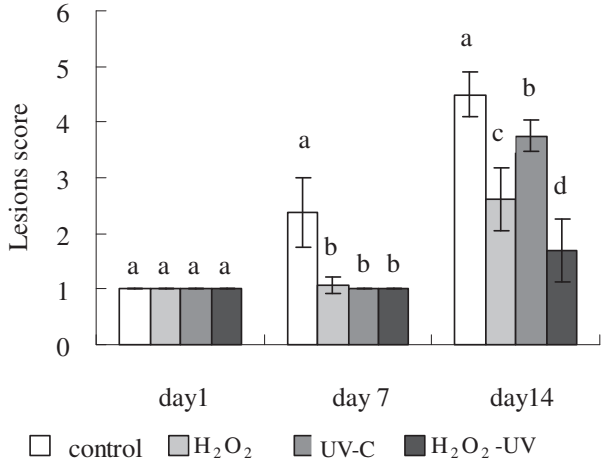

Fig. 3. Effect of H_2O_2 and UV-C on lesions of button mushroom stored for 14 days at 4 °C. Vertical bars represent standard deviation ($n = 4$). Means with different letters on same day are significantly different ($P < 0.05$).

Table 2
Effect of different treatments on total phenolics and ascorbic acid of button mushrooms during 14-day storage at 4 °C.

	1 day	7 days	14 days
	Total phenolics content (mg Gallic acid equivalent/100 g f.w.)[a]		
CK	49.80 ± 2.76b	56.31 ± 3.08b	70.75 ± 4.70a
H_2O_2	48.64 ± 2.89b	58.53 ± 3.69b	76.83 ± 13.15a
UV	52.36 ± 2.60ab	57.56 ± 4.85b	69.05 ± 3.51a
H_2O_2 + UV	56.06 ± 6.41a	64.54 ± 2.71a	74.48 ± 9.42a
Treatment		***	
Storage time		***	
Treatment × storage time		*	
	Ascorbic acid (mg/100 g f.w.)[a]		
CK	0.078 ± 0.021a	0.132 ± 0.027a	0.128 ± 0.003a
H_2O_2	0.072 ± 0.032a	0.110 ± 0.040a	0.129 ± 0.022a
UV	0.087 ± 0.040a	0.124 ± 0.063a	0.136 ± 0.015a
H_2O_2 + UV	0.076 ± 0.026a	0.113 ± 0.038a	0.131 ± 0.016a
Treatment		NS[b]	
Storage time		***	
Treatment × storage time		NS[b]	

[a] Means followed by the same letters within same column are not significantly different ($P < 0.05$).
[b] NS, *, ** and *** indicate no significance or significance of treatments, storage days and interaction between treatments and storages days at $p < 0.05$, 0.01 and 0.001 levels, respectively.

The highest value of total phenolics of all the treated mushrooms was detected in mushrooms treated with H_2O_2 + UV during storage. H_2O_2 + UV treated mushrooms had significantly higher total phenolics than the control and the single treated samples at days 1 and 7, while the significant difference among treatments disappeared at day 14. Ascorbic acid content was not significantly ($p > 0.05$) affected by any treatment during storage.

Sapers and Simmons (1998) reported that 5% H_2O_2 wash followed by a spray application of 4% sodium erythorbate solution resulted in greater retention of soluble phenols, both in the pileus and stipe, than the water washed or unwashed mushrooms. Several reports have indicated that UV-C irradiation increased ascorbic acid content in shiitake (*Lentinus edodes*) mushrooms and broccoli, while a decrease in ascorbic acid by UV-C has also been reported in fruits and vegetables (Jiang et al., 2010; Lemoine, Civello, Martínez & Chaves, 2007). Overall, our results showed that the combination of H_2O_2 and UV-C would not cause the decrease in phenolics and ascorbic acid contents of button mushrooms.

4. Conclusions

H_2O_2, UV-C and H_2O_2 + UV treatments could marginally effective in reducing *E. coli* O157:H7 population, or native microbial loads, while the treatments reduced lesion development on button mushrooms during 14 days' storage at 4 °C. Mushrooms treated

with UV-C initially developed more browning than the control samples, although the browning occurred less rapidly during storage. H_2O_2 and H_2O_2 + UV were better in maintaining the white color of mushrooms than other treatments. H_2O_2 + UV could extend storage periods with little deterioration of visual and nutritional quality of button mushrooms. The combined treatment was more effective than any single treatment in inhibiting lesion development. Overall, the results indicate that the combination of H_2O_2 and UV-C would be a potential postharvest treatment for maintaining the quality of button mushrooms.

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